

Applicant : Neil H. Bander et al.
Serial No. : 09/655,708
Filed : September 6, 2000
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Attorney Docket No.: 10448-185002 / MPI1999-
325P1RCN1

In the Specification:

Please amend the title at page 1, line 1 as follows:

A METHOD FOR ISOLATION OF PROSTATIC EPITHELIAL CELLS FROM
SEmen-A SOLUTION

Please amend the section entitled "Related Application Data" at page 1, lines 3-4 as follows:

This application is a Continuation of United States Serial No. 09/655,708, filed September 6, 2000, and claims priority from application United States Serial No. 60/153,506 filed September 13, 1999, both of which are incorporated herein by reference in their entirety.

Please amend the section entitled "Brief Description Of The Drawings" beginning at page 7 line 20 through page 8, line13 as follows:

Fig. 1 shows the enrichment of tumor cells by magnetic activation cell sorting, using the method of the present invention. A total of 5x10⁴ LNCaP cells were mixed with semen (LNCaP to sperm ratio was 1:34). Fig. 1A—Before MACS, there were many sperm admixed with LNCaP cells. Fig. 1B—After separation, most sperm have been removed from the semen. The cells were stained with Papanicolaou stain. Prostasomes, presumably positive for PSMA, were also concentrated in this technique. (Magnification, 400x).

Legend:

-  — Squamous Epithelial Cell
-  — Spermatozoa
-  — Degenerating LNCaP Cell

Fig. 2 shows FIGS. 1A-C show flow cytometric analysis of semen spiked with cultured prostate cancer cells (LNCaP) before and after treatment according to the method of the present invention. The cells were passed down a magnetic activated cell sorter (MACS) separating column. Fig. 2A 1A - Cells before separation. Fig. 2B 1B - the positive fraction eluted after removal from magnetic field. Fig. 2C 1C - the negative fraction which flowed through the column. Regions Region R1 contains prostasomes and cell fragments; Region R2 contains PSMA positive prostate epithelial cells; and Region R3 contains mature spermatozoa.

Fig. 3 represents a cytopsin preparation of MACS-enriched and flow cytometry sorted cells. Figures 3A, 3B, and 3C represent sorted cells from Regions R2, R2, and R3 of Fig. 2, respectively. The cells were stained with Papanicolaou stain. Magnification, 400x.

Please amend the paragraph titled "3. Flow cytometry and cytologic analysis of MACS isolated cells" beginning at page 11, line 18 as follows:

A total of 5×10^4 LNCaP cells were mixed with semen (LNCaP to sperm ratio was 1:34). The collected positive fractions and negative fractions were assessed by conventional cytology (the cells were stained with Papanicolaou stain) (Fig. 1) and flow cytometry (Fig. 2 1). Before MACS, there were many sperm admixed with LNCaP cells. After separation, most sperm had been removed from the semen. Prostasomes, presumably positive for PSMA, were also concentrated in this technique. For the flow cytometry analysis, the forward scatter (size) versus side scatter (granularity) graph was used to include all cellular elements. Events gated were then represented on a two dimensional log graph. For fluorescence sorting, the propidium iodide staining for DNA was represented horizontally, and PSMA staining was represented vertically. As shown in Fig. 2B 1B, the PSMA positive cell purity of positive fraction was about 40-50% by flow cytometry. By cytologic evaluation (Fig. 3), the R1 region was found to be prostasomes and dead cell fragments (Fig. 3A). An increased percentage of events in R1 after column sorting can be explained by mechanical damage during the multistep procedure. As shown in Fig. 3B, by dual action of MACS and flow cytometric sorting, sorted cells from R2 were nearly pure LNCaP cells. The R4 region was hypothesized to be PSMA-negative intact cells, such as

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leukocytes, but due to low cell count in normal semen, we could not confirm these components.

As shown in Fig. 2C 1C, there were very few tumor cells in the negative fraction.